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The fine structure of the house of appendicularians
(Oikopleura dioica Fol.) and of the tunic of
ascidian larvae (Botryllus schlosseri Pall.)

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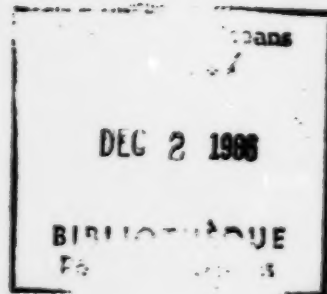
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From the Zoological Institute (Directors Prof. Dr. W. Ulrich and Prof. Dr. K. Herter) and the Department of Electron Microscopy of the Faculty of Mathematics and Natural Sciences (Director Prof. Dr. E. Ruska) of the Freie Universität Berlin (Free University of Berlin).

The Fine Structure of the House of Appendicularians (Oikopleura dioica Fol.) p. 431
and of the Tunic of Ascidian Larvae (Botryllus schlosseri Pall.)

Georg Kümmel

With 9 Figures

Introduction

Function and development of the house of the appendicularian genus Oikopleura are well known through the works of Lohmann (1909, 1933) and Körner (1952), who also discusses the chemical composition of the house. However, the fine structure of the house still requires clarification and this will be attempted in the following study.

The appendicularians are often placed in affinity with the tailed ascidian larvae. The question arises whether this affinity extends also to the sub-microscopic range. For comparison purposes, I will therefore describe below the fine structure of the tunic of the larvae of Botryllus schlosseri Pall.

Material and Methods

The only material available for the studies were fixed specimens obtained from the Heligoland Station. Oikopleura dioica Fol. was fixed with mercuric chloride/glacial acetic acid and subsequently preserved in 70% alcohol. Mercuric chloride/glacial acetic acid is certainly not a particularly suitable fixative for electron microscopic studies. However, some, although very few, specimens which had been fixed solely with Formol were also available for study. Since the results were basically the same, it can be assumed that the mercuric acid/glacial acetic acid fixative had no adverse effect on the membranes of the Oikopleura house⁵.

More than 60 specimens were examined. The houses enclosing the bodies were separated from the latter. Of course, the development stages of the houses varied. The houses were mounted on slides either without treatment or after treatment with concentrated nitric acid at different temperatures (see legends for the various figures). p. 432

The larvae of Botryllus (more than 50 specimens examined) were fixed in Formol and stored. The "cuticles" (see below) were removed and mounted on slides without further treatment, while the tunic proper was cleaned with potassium hydroxide solution (10%) or chromic acid (10%). The specimen slides were not coated.

An Elmiskop II^{*} operated at 60 kv was used for the studies. In addition, the findings from the electron microscope studies were verified in a phase-contrast and a polarizing microscope. The chemical composition of the tunic was determined only in the ascidian larvae. The usual test for cellulose was carried out with iodine and sulfuric acid and the Hotchkiss reaction was used to test for polysaccharides or glycoproteins.

*Translator's note: An electron microscope manufactured by Siemens.

A. Oikopleura dioica Fol.

The house of Oikopleura is not a uniform structure, as has been known for some time. I need only refer to the above-mentioned works by Lohmann and Körner. Of course, the incurrent grids and the feeding apparatus are morphologically the most interesting structures. Unfortunately, the feeding apparatus is too thick to examine it in a normal transmission electron microscope. However, the fine structure of the membrane per se had to be determined first. Lohmann (1933) has illustrated fibrils in the house which, in transverse sections, are perpendicular to the membrane surface. Körner (1952), on the other hand, provides instructive illustrations of moniliform secretory strands which extend between two boundary membranes in the plane of the house and have a diameter of 0.5 to 1 μ .

Our studies using the electron microscope produced the following results: Fibrillar units appear even without further treatment, although they are not very distinct. On the other hand, even a short treatment with nitric acid reveals a very clear and delicate fibril pattern which is extremely regular in certain, not precisely identifiable spots (Fig. 1)¹⁾. These fibrils have a diameter of about 200 Å. Thus, I have not been able to find moniliform secretory strands. It should be mentioned though, that Körner's figures represent the stage shortly after secretion of the membrane and after staining. This may explain the discrepancy between the two findings. That the fibrils are perpendicular to the surface, as Lohmann suggests, is improbable to say the least and, moreover, hardly worth discussing since he fails to indicate their size in his drawing.

¹⁾ The figures reproduced in this paper are not as sharp and convincing as the originals deposited in the Fritz-Haber Institut of the Max-Planck Gesellschaft (Department of Prof. Dr. E. Ruska).

It is conceivable that the fibrils I observed are the result of drying and denaturing of a previously structureless membrane. However, this is unlikely since a first trace of the fibrils can already be seen in the phase contrast microscope and specifically the incurrent grids, which will be discussed in more detail below, show a fibril structure when viewed in the polarizing microscope.

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The two incurrent grids have a considerably more complex structure, as seen already under the optical and polarizing microscope. Large cells form a gelatinous cushion which serves as a support for the primary and secondary fibrils secreted by small cells and which subsequently undergoes cell reduction.



Fig. 1. Membrane of the house of *Oikopleura dioica*. Uniform fibril texture. Treatment: concentrated nitric acid, 1 hr. 55°C. Electron microscope, x 7600, subsequently enlarged.

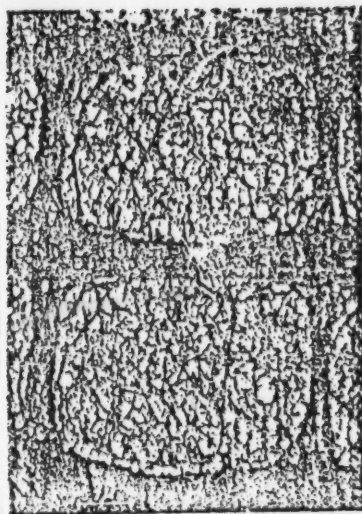


Fig. 2. Incurrent grid of Oikopleura dioica. The pronounced grid structure corresponds to the network which is visible in the microscope. Fibrillar gelatinous cushion underneath. Treatment: concentrated nitric acid, 1 min., 20°C.



Fig. 3. Incurrent grid of Oikopleura dioica. Secondary fibrils spreading through the primary fibrils. Treatment: concentrated nitric acid, 5 min., 60°C.

The fine structure of the elements which Körner was unable to see with the technology available at that time can now be discerned in the electron micrograph. The primary and secondary fibrils are composed of still more delicate microfibrils and not of a string of secretory granules as described by the above-mentioned author for Oikopleura albicans Leuckart. The microfibrillar structure of the primary and secondary fibrils is particularly pronounced in houses which have been spread out and treated very little (Fig. 2). Here the micrograph shows the gelatinous cushion which is likewise fibrillar.

Körner states that the transverse fibrils merge with the primary fibrils.

However, my micrographs suggest that the transverse fibrils are formed as a whole since they spread through the primary fibrils (Fig. 3). Apparently, the transverse fibrils are generally arranged in groups of two or three in a continuous membrane, which is certainly not identical with the cover membrane, p. 435 but perhaps with the above-mentioned boundary membrane (Fig. 4). Subsequently, these groups of fibrils separate and form the boundaries of the grid orifices, thereby tearing the continuous membrane (Fig. 5).

In one incurrent orifice I noticed that fibrils had developed in one direction only and that these fibrils in turn were lying in a continuous membrane (Fig. 6). In my view, these are the primary fibrils of an incurrent orifice which is still in the developmental stage, and demonstrate that the two types of fibrils are formed successively. Yet I cannot explain how the subsequent, close interconnection occurs between the two types of fibrils.

The micrographs require some additional explanations. The isolated houses were placed over the apertures of the specimen slides without carrier foil and then

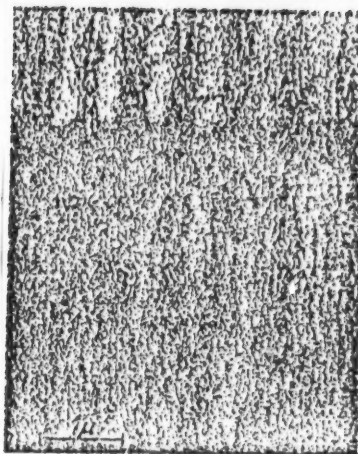


Fig. 4. Incurrent grid of *Oikopleura dioica*. Transverse fibrils in groups of three. Treatment: concentrated nitric acid, 5 min., 60°C, electron microscope, x 7600, subsequently enlarged.

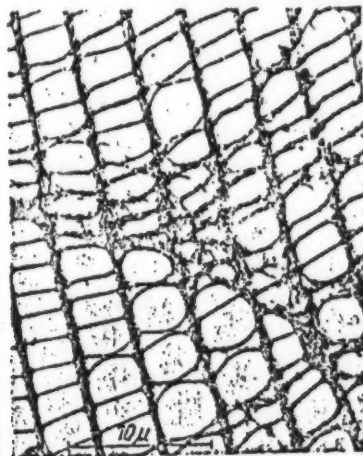


Fig. 5. Incurrent grid of *Oikopleura dioica*. Separation of secondary fibrils. Treatment: concentrated nitric acid, 5 min., 60°C, electron microscope, x 1300, subsequently enlarged.

dried, as was described in the section dealing with materials and methods. Of course, this procedure produces stresses, particularly in houses which have been widely spread out, and these stresses may enlarge, and sometimes even distort, the openings of the mesh. Yet the method has the advantage that the fibrils appear more distinctly. p. 436

Some of the micrographs show rod-shaped elements along the fibrils (Fig. 6). These rod-shaped elements are certainly mercury salt crystals from the mercuric chloride fixative which have become embedded along the lines defined by the fine structure of the fibrils. Perhaps this unexpected finding may be used to develop a method for staining protein fibres. However, I have not made any systematic investigations in that regard.

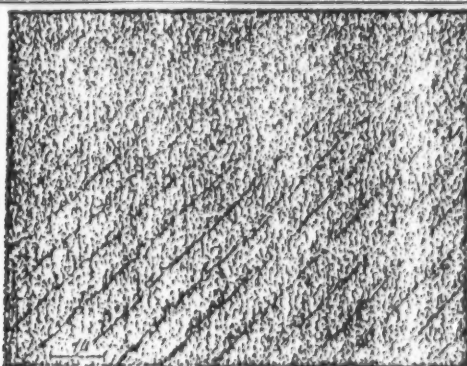


Fig. 6. Incurrent grid of Oikopleura dioica. Only the primary fibrils are developed. Marked by embedded mercury salt crystals. Treatment: concentrated nitric acid, 30 min., 20°C; 2 min., 60°C.

B. Botryllus schlosseri Pall.

The cellulose of imaginal ascidians (the so-called tunicin) has previously been studied by several authors using electron microscope techniques (Frey-Wyssling and Frey 1950, Meyer, Huber and Kellenberger 1951, Rånby 1952). However, the nature of the material requires prior mechanical treatment with ultrasound or blender, in addition to chemical cleaning, before an electron

beam can penetrate them, which will then reveal networks of microfibrils with a diameter of 100 to 200 Å. According to some authors (Mayer, Huber and Kellenberger, 1951), the microfibrils are ribbon-shaped. On the whole, they closely resemble specimens obtained from plant cellulose. However, several authors emphasize that the ribbon-shaped tunicin microfibrils are frequently fused to form flat aggregates, in contrast to the microfibrils in the cell walls of plants. This characteristic is said to be associated with the somewhat better orientation of the fibrils in the X-ray diffraction pattern (Fry-Wyssling and Frey, 1950).

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The tunics of the ascidian larvae do not require any mechanical preparation before they can be studied under the electron microscope. Chemical cleaning is sufficient to provide a fairly accurate picture of the original fibril textures. Bars clearly composed of microfibrils cover the tunic at intervals of several microns (Fig. 7). Since these bars intersect and are interwoven, they form many windows between them which are covered by a dense network of microfibrils. These fibrils, which have the usual diameter of 100 to 200 Å, are in turn interwoven in a relatively irregular fashion (Fig. 8). In many instances, however, these microfibrils are seen to be closely spaced to form aggregates, which may very well correspond to the above-mentioned ribbon-shaped, fused aggregates. The bars may also be regarded as such aggregates since they, too, are flattened along the tunic plane, as can be seen from the stereographs.

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Fig. 7. Cellulose tunic of Botryllus larva. Network of bars and microfibrils. Treatment: chromic acid (10%), 36 hrs., 20°C, electron microscope, x 1300, subsequently enlarged.



Fig. 8. Cellulose tunic of Botryllus larva. Network of microfibrils. Treatment: chromic acid (50%), 1 hr., 20°C, electron microscope x 15400, subsequently enlarged.

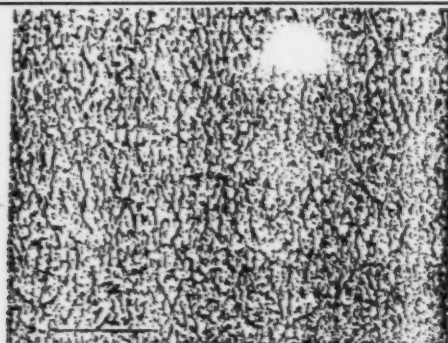


Fig. 9. Cuticle of Botryllus larva. Secretory strands. Untreated. Electron microscope, x 7600, subsequently enlarged.

However, the cellulose tunic forms only part of the entire tunic. It consists of two layers in the larvae of Botryllus, namely an inner layer which I have described above and which I regard as tunic proper, and an outer layer which I call cuticle. This cuticle appears in the optical microscope as a completely clear, transparent and homogeneous epidermis. The polarizing microscope shows a weak birefringence of the cuticle when viewed on edge only when the Brace compensator is used. The normal test for cellulose with sulfuric acid and iodine is completely negative. However, the Hotchkiss reaction (according to Lipp, 1954) is positive, although rather weak. It indicates that the membrane does not contain cellulose but may well consist of a glycoprotein, as does the house of Oikopleura (Körner, 1952).

The electron micrograph of the membrane differs considerably from that of the cellulose tunic. It should be mentioned, however, that the membrane has not been treated since all previous treatments applied to membranes destroyed them. Strands of several 100 Å diameter appear in an inhomogeneous matrix (Fig. 9). A moniliform structure of these strands may be discerned in some instances.

I am not certain about the homology of the cuticle. Although Grave and Woodbridge (1924) have described the morphology of the larva of Botryllus schlosseri Pall., they do not mention the cuticle. It may well be that this cuticle is homologous to the cuticle of adult ascidians which covers the tunic proper in the form of a usually thin membrane. However, this cuticle is said to contain some, albeit little, cellulose (Saint-Hilaire, 1931).

Conclusion

The fine structure of the house of Oikopleura, an appendicularian, and of the tunic of Botryllus schlosseri Pall., an ascidian, has been studied. The frequently claimed homology between these two structures is not discernible, at least as far as the fine structure is concerned. The chemical composition of the tunic proper of the ascidian larvae is also completely different from that of the appendicularian house. On the other hand, the cuticle of the ascidian larvae has a chemical composition comparable to that of the house of the appendicularians.

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